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Sex increases the probability of evolutionary rescue in the presence of a competitor

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Abstract

The explanation for the continued existence of sex, despite its many costs, remains one of the major challenges of evolutionary biology. Previous experimental studies have demonstrated that sex increases the rate of adaptation in novel environments relative to asexual reproduction. Whilst these studies have investigated the impact of sex on adaptation to stressful abiotic environments, the potential for biotic interactions to influence this advantage of sex has been largely ignored. Species rarely exist in isolation in natural conditions, so the impact of sex on adaptation to a stressful abiotic environment may be altered by the interactions between coexisting species.

To investigate the interplay of sex and competition on adaptation to deteriorating conditions, we allowed populations of the unicellular alga (*Chlamydomonas reinhardtii*) to evolve in an environment to which they were initially poorly adapted. We manipulated both their mode of reproduction and the presence of a competitor, and monitored population size and proportion of evolutionary rescue events for each mode of reproduction.

The results indicate that sex may be the beneficial strategy in the presence of the competitor. Sexual populations had highest probability of evolutionary rescue irrespective of the presence of the competitor. The overall advantage of sex was also manifested through higher level of adaptedness of survived sexual populations relative to asexual populations. Since competitive interactions are commonplace in nature, one of the explanations for the maintenance of sex by natural selection may be the increased rate of adaptation of sexual populations both in the presence and absence of competitors.

Key words: *Chlamydomonas reinhardtii*, sex, competition, evolutionary rescue

1. Introduction

The great majority of extant eukaryotic species are sexual (Bell, 1982). Despite its ubiquity, sex still remains an unresolved challenge for evolutionary biologists to explain. This is why sex has been a subject of numerous theoretical and experimental studies for more than a century (Kondrashov, 1983; Burt, 2000; Hartfield and Keightley, 2012).

The paradox of sex is reflected in the fact that it entails various costs that are disadvantageous for an individual and a population. For example, sex may result in a decline of mean fitness of a population as a result of a break-up of favorable combinations of alleles, termed recombination load (Barton and Charlesworth, 1998), as experimentally demonstrated (for example: Greig et al., 1998; Colegrave et al., 2002; Kaltz and Bell, 2002; Becks and Agrawal, 2012). In addition to such genetic costs, sex can impose direct costs as well, such as investment of resources into finding a mate or increased likelihood of transmission of pathogens and parasites between individuals or their gametes. Moreover, in anisogamous species, sex entails the two-fold reduction of reproductive output per female as a cost of producing males, which has been termed “the two-fold cost of sex” or “the cost of males” (Maynard Smith, 1971; Williams, 1975; Maynard Smith, 1978). Considering all these costs, the continued existence of sex implies that there must be a compensatory selective advantage.

Most hypotheses that explain the benefits of sex gravitate towards the idea that sex generates variation within a population, which is then available for selection to act on, and thus enhances adaptation to a changing environment (Weismann, 1889; Fisher, 1930; Muller, 1932; Felsenstein, 1974; Burt, 2000). The major advantage of sex based on this concept is faster accumulation of multiple beneficial mutations within a single individual, which in turn increases the response of natural selection and the rate of adaptation. In contrast, the fixation of beneficial mutations would proceed at a slower rate (one at a time) in asexual populations, which would ultimately result in the competition of genotypes carrying

different mutations (Muller, 1932), consequently slowing down adaptation (a process termed clonal interference; Gerrish and Lenski, 1998).

Recent experimental work has shown that sex can have important consequences for the rate at which a population adapts to novel environments. For instance, Kaltz and Bell (2002) subjected experimental populations of *Chlamydomonas reinhardtii* to heterotrophic growth on novel carbon sources and compared the rate of adaptation of sexual and asexual populations. Repeated episodes of sex provided a long-term advantage in mean fitness relative to asexual populations. This relative advantage of sexual populations is directly proportional to population size prior to induction of sex, as demonstrated by Colegrave (2002). This result suggests that sex reduces the constraint of adaptation imposed by clonal interference in larger populations. Becks and Agrawal (2012) subjected sexual and asexual populations of a monogont rotifer *Brachionus calyciflorus* to novel environments (elevated concentration of NaCl and novel food source) and found that during the initial stages of adaptation sexually derived offspring had higher fitness (measured as lifetime reproduction per female) than the asexually produced genotypes. In addition to more efficient adaptation, sex may decrease the likelihood of extinction. Bell (2013) found that the populations of *C. reinhardtii* with a history of obligate sexual reproduction have higher probability of survival relative to asexual populations, when subjected to growth in the absence of light.

A growing body of experimental studies demonstrates that sex is also beneficial in deteriorating environments. Goddard et al. (2005) compared the rate of adaptation between sexual and asexual yeast populations in benign and deteriorating conditions (an increase of osmolarity and temperature). Relative fitness of sexual populations (measured as growth rate) was significantly higher in the deteriorating environment, but not in benign conditions. Sex may also increase the likelihood for survival of a population in conditions that would have ultimately caused extinction of an ancestral population, a process termed “evolutionary rescue” (Gomulkiewicz and Holt, 1995). Lachapelle and Bell (2012) manipulated genetic diversity and mode of reproduction (obligate sexual, facultative sexual and asexual) of *Chlamydomonas reinhardtii* populations, and monitored the extinction dynamic while the

environment gradually deteriorated (an increase of salinity). They found that a combination of obligate sexuality and high genetic diversity significantly increased the survival rate. Furthermore, high-diversity sexual populations had significantly higher level of adaptation to high salt concentrations relative to other treatment groups.

Previous experimental studies have focused on the impact of sex on adaptation to a stressful abiotic environment. However, these studies have largely ignored the potential for biotic interactions to influence the advantage of sex. Investigating the effect of sex within a multispecies context is essential, because in natural conditions species rarely exist in isolation. Hence, the ability to adapt and the effect of sex on adaptation may depend on interactions between coexisting species.

The effects of sex in the presence of other species have been mostly studied within a parasite-host system. Lively (1992) found positive correlation between the frequency of males in a facultative sexual species *Potamopyrgus antipodarum* and the presence of parasites (trematodes). Morran et al. (2009) provided experimental evidence for increased level of outcrossing in the wild-type population of *Caenorhabditis elegans*, when subjected to a bacterial pathogen (*Serratia marcescens*). However, while these studies have been focused on the biotic factors which increase the frequency of sex, to our knowledge, no experimental study has investigated whether the effects of sex on adaptation are altered by the presence of other species.

Here, we focus on how competitive interactions may modulate any effects of sex on adaptation. We focus on the competitive (negative) interactions between species, because recent studies (Johansson 2008; Collins 2011) demonstrated that competition restricts adaptation and so might be predicted to constrain the beneficial effects of sex. Johansson (2008) modeled competition between two species in a changing environment and found that the species disfavored in competition suffers a decline in effective population size, which in turn reduces the maximal rate of adaptation. This result was corroborated by experimental study of Collins (2011). She demonstrated that the rate of adaptation (absolute fitness) of *Chlamydomonas reinhardtii* to elevated concentration of CO₂ declines in presence of a

competitor, due to a trade-off between adaptation to abiotic and biotic component of environment.

Interplay of sex and competition may result in several outcomes. Firstly, the beneficial effect of sex may increase with an increase of complexity of environment (incorporation of a competitor into the environment of a focal species). Adaptation to complex environments may involve multiple loci (Kaltz and Bell, 2002). Under these conditions, sex may allow for more efficient assembling of multilocus genotypes, thus increasing the fitness advantage of sexual over asexual populations (Kaltz and Bell, 2002). However, the presence of a competitor is likely to cause a decline of population size of adapting population, thus reducing the supply of beneficial mutations. Consequently, the rate of adaptation may decline irrespective of mode of reproduction of a population. Under these conditions, sex may still remain the beneficial strategy, but relative advantage of sexual populations (an increased rate of adaptation) diminishes. Alternatively, sex may be disfavored if negative effects of competition are augmented by the genetic costs such as recombination load. A decline in population size caused by competition, coupled with a short-term decline of mean fitness of a population may outweigh the benefits of sex and thus favor asexual mode of reproduction.

In order to investigate the combined effect of sex and competition on adaptation to changing (deteriorating) conditions, we allowed populations of the unicellular green alga (*Chlamydomonas reinhardtii*) to evolve in an environment to which they were initially poorly adapted. Under these conditions, the populations decline and, in the absence of evolutionary change, the populations will go extinct. We manipulated both their mode of reproduction (either entirely asexual, or with a history of sexual reproduction) and the presence of a competitor. We monitored population size and proportion of surviving populations (the number of evolutionary rescue events) for each mode of reproduction throughout the course of experiment.

If sex is beneficial in both the presence and absence of a competitor, we predicted a higher number of evolutionary rescue events in sexual populations, irrespective of the competition treatment. If the advantage of sex is more pronounced in the presence of the competitor, the relative proportion of evolutionary rescue events of sexual populations will increase in the presence of the competitor. Finally, if sex is disadvantageous in the presence of the competitor, we predicted a lower proportion of evolutionary rescue events relative to asexual populations.

2. Materials and methods

Base Populations of Chlamydomonas reinhardtii

In order to establish genetically variable experimental populations of the focal species, *C. reinhardtii*, the mass mating of 10 different wild types strains was performed. The zygotes obtained from the mass mating were plated on agar and allowed to germinate by producing zoospores. The zoospores differentiated into adult vegetative cells which were allowed to undergo several rounds of mitotic divisions until visible colonies appeared on agar plates. Each colony represents a single (unique) genotype derived from an individual cell. *C. reinhardtii* is an isogamous organism with two mating types (mt): mt+ or mt- and no mating type switching. A library of 40 genotypes was established by randomly picking 20 colonies of each mating type from the agar plates by sterile loops. The mating type was determined by crossing the culture derived from each genotype with tester isolates. Each experimental population represents a unique combination of 10 genotypes, randomly chosen from the library: 48 sexual populations were established by combining 5 mt+ and 5 mt- isolates; two sets of 24 asexual populations were established by combining 10 mt+ and 10 mt- isolates, respectively.

The Competitor Species (Chlamydomonas moewusii)

The competitor species used in this study was another unicellular chlorophyte alga, *Chlamydomonas moewusii*. This freshwater species was selected for the experiment due to diversity of strains isolated from natural habitats and similar growth requirements as *C. reinhardtii*. We obtained 4 wild type strains (cc-1419, cc-1420, cc-1480 and cc-1481) from the University of Minnesota algae collection (<http://www.chlamycollection.org/strains/>). All wild types of the competitor species were cultivated separately throughout the course of experiment, hence no rounds of sexual reproduction have been allowed.

EXPERIMENTAL EVOLUTION

Experimental evolution was carried out in two phases. In the first phase, populations of the focal species experienced a deteriorating environment in which NaCl (hereafter referred to as salt) concentration increased to levels that were expected to be lethal. In the second phase, their ability to adapt to these conditions was monitored in both the presence and absence of the competitor species.

Cultivation and Transfer Procedure

All experimental populations of *C. reinhardtii* were cultivated in 24-well plates under standard conditions (26°C, 3200 lux illumination, shaking at 180 rpm and covered with sterile breathable membranes to prevent cross-contamination and uneven evaporation across the plates). A serial passage was performed after every 3-4 days (a period hereafter referred to as growth cycle, corresponding to approximately 9-12 doublings of cells per culture) by transferring 5 % of each population to a fresh medium. After every second growth cycle,

each population was sampled (150 µl) and population size estimated spectrophotometrically by measuring optical density (OD₇₅₀) of the culture. The experimental populations were propagated in Bold's broth medium (2 ml per culture) supplemented with salt, which concentration gradually reached 15 g/l in three equal steps of increase (5 g/l). The salt concentration of 15 g/l was chosen as an endpoint because it completely inhibits the growth of *C. reinhardtii* (Reynoso and de Gamboa, 1982). The stepwise increase in salt level was selected as an experimental procedure since the immediate exposure to the lethal stress would have likely resulted in a too abrupt decline of population density. The likelihood of contact between two opposite gametes decreases in a sparse population which can consequently hamper the completion of the sexual cycle (production of zygotes).

Sexual Cycle

Prior to each step of salt increase (after every two growth cycles), sexual populations were allowed to undergo a sexual cycle. We allowed three sexual cycles in total, since Kaltz and Bell (2002) had demonstrated that three successive episodes of sex provide a long-term increase of the rate of adaptation of sexual populations. Each sexual cycle was induced using the following protocol. All the populations were centrifuged at 5000 rpm for 10 minutes, re-suspended in nitrogen-free medium to initiate gametogenesis and incubated in standard conditions under bright light for another 24 hours to allow for mating and formation of zygotes. After given period, the plates containing experimental populations were wrapped in aluminium foil and incubated in dark for additional 4-5 days to allow the zygotes to mature. Since sublethal stress may affect the mutation rate, the effects of sex may be confounded with the effects of mutations (Goho and Bell, 2000; Colegrave et al, 2002). Hence, the nitrogen starvation treatment was also applied to asexual populations, but since they were made up of single mating types, no mating took place. After incubation in darkness, the plates with mature zygotes were placed in a freezer for 4 hours (-20°C) in order to eliminate

unmated gametes. The zygotes develop the additional layers of cell wall during maturation in the darkness, thereby acquiring resistance to stressful conditions (Harris, 2009). The pilot experiments in our laboratory revealed that this feature enables the zygotes to withstand low temperature. Given the lack of resistance of unmated gametes to freezing, this procedure was omitted for the asexual populations. The zygotes of the sexual *C. reinhardtii* populations were then transferred to agar plates by sterile loops and incubated in bright light for two days to allow for germination and several rounds of mitotic divisions. The asexual *C. reinhardtii* populations were transferred to agar plates by pipetting (an aliquot of 150 μ l) and incubated for the same interval of time. After given period, all the cultures in agar plates were flooded with 4 ml of Bold's broth medium (supplemented with salt which concentration increased for 5 g/l than prior to induction of sexual cycle) for approximately an hour. Population size of each experimental population on agar was estimated spectrophotometrically (OD₇₅₀) and diluted to the same optical density as prior to induction of sexual cycle. This experimental procedure ensured that effects of sex were not confounded with a variation in population size. All the populations were then returned to the liquid medium.

Assay of high salt tolerance of both species

The probability of evolutionary rescue of the focal species might be affected by the potential differences in high salt sensitivity between *C. reinhardtii* and *C. moewusii* since the species with a faster growth rate in the high salt environment (and therefore, more tolerant to the stressful conditions imposed on both competitors) could reduce population size of the species with a slower growth rate (relative to the treatment without the competitor). Since the probability of evolutionary rescue is positively correlated with population size (Bell and Gonzales, 2009), the populations of the less tolerant species may adapt less efficiently and ultimately go extinct. To investigate this possibility, all 40 ancestral isolates of experimental *C. reinhardtii* populations and all four *C. moewusii* wild type isolates were assayed for growth

in the high salt medium (Bold's supplemented with 15 g/l of salt) prior to commencing of the experiment. The populations of both species were allowed two growth cycles in 96-well plates (3-4 days; 5% of culture passaged), but were assayed only during the second growth cycle, to avoid the possible carry-over effects after plating from agar slant tubes, used for storing the cultures. The growth rate (average yield per time point) was estimated spectrophotometrically, by measuring optical density (OD₇₅₀) twice a day (approximately every 12 hours), until the cultures reached the stationary phase (after approximately 4 days).

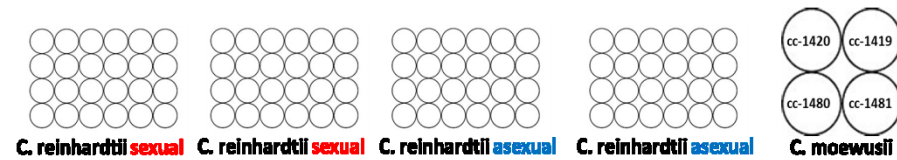
Establishing and Cultivation of Mixed Populations

To examine the interplay between mode of reproduction and competition, during the second phase of the experiment we manipulated the presence or absence of the competitor (summarized in Figure 1, see below). The decision not to add the competitor to the first phase of experiment was taken, because the freezing procedure applied during the sexual cycle of *C. reinhardtii* would have been lethal for *C. moewusii*. In the second phase of the experiment, at the point which the environment had reached its maximum salt concentration, the mixed populations were assembled by combining each wild type of *C. moewusii* with six sexual populations and six asexual populations of *C. reinhardtii*. The isolated asexual and sexual *C. reinhardtii* populations (twenty-four per each mode of reproduction) were not combined with the competitor. Prior to assembling with *C. reinhardtii* populations, the populations of all four *C. moewusii* wild types underwent the same cultivation and transfer procedure as described (see "Cultivation and Transfer Procedure" section), including the three rounds of salt increase.

Both species of the mixed populations started this phase of the experiment with equal cell densities, estimated the following way. Nine randomly chosen populations of *C. reinhardtii* and the population of each wild type of *C. moewusii* were sampled (150 µl) and population size estimated by cell counting with a haemocytometer. Each of the *C. moewusii*

wild type populations were then diluted to the culture density corresponding to the average cell number of the sampled *C. reinhardtii* populations.

A) First phase of experiment



B) Second phase of experiment



Figure 1 – Experimental design. In the first phase of experiment, which lasted until salt concentration reached 15 g/l, both species were cultivated separately: two groups of sexual populations of *C. reinhardtii*, two groups of asexual populations of *C. reinhardtii* and a single population of each wild type of *C. moewusii*; each circle represents a single population. During this phase, populations in sexual groups of *C. reinhardtii* underwent three sexual cycles. In the second phase, each wild type of *C. moewusii* was combined with six populations of one sexual *C. reinhardtii* group and six populations of asexual *C. reinhardtii* group. The remaining sexual and asexual groups of populations were propagated in isolation, without the competitor.

All the mixed populations and control *C. reinhardtii* populations were cultivated in the Bold's broth medium supplemented with 15 g/l of salt throughout the rest of experiment, maintained by serial passage (5 % of each population) performed after every 3-4 days. Prior to each passage to the fresh medium, a sample (200 µl) of each population was serially diluted (10^{-1} or 10^{-2} - fold dilution) and transferred to the corresponding Petri dish with Bold's agar medium supplemented with sodium-acetate. Each sampled population was incubated until colonies appeared (approximately 4 days) and the cell number was estimated by colony

counting. The cell number of the population sample was converted to the cell number (per ml) of the whole population by using the equation: cell number per ml = number of colonies / dilution factor X sample size (0.1 ml). After given interval of time, all the mixed populations were wrapped in aluminium foil and incubated in dark for additional 3-4 days to determine the population size of each species.

Distinguishing the two species

To distinguish the two species, we grew them under conditions in which they would show different growth characteristics on agar plates. We made use of the fact that *C. reinhardtii* grows well in the dark on acetate supplemented agar plates, whilst *C. moewusii* does not.

Both species are facultative heterotrophs, capable of utilizing sodium-acetate while incubated in light. However, *C. moewusii* cannot metabolize sodium acetate in the dark or otherwise shows poor growth (Harris, 2009). In contrast, *C. reinhardtii* cells utilize sodium acetate and continue dividing in the dark, although with a reduced growth rate than under light conditions. This biological feature had been exploited in this experiment, given that *C. reinhardtii* colonies clearly increased in size after incubation in darkness, thus becoming distinguishable in a mixed culture. After the identification based on colony sizes, colonies were additionally inspected (under inverted microscope with 20 times magnification) to distinguish the two species based on the individual cell features. The cells of *C. reinhardtii* are oval or spherical, while the *C. moewusii* colonies comprise the elongated and ellipsoid cells (personal observation).

Recording the evolutionary rescue events

Experimental evolution was continued until all *C. reinhardtii* populations either went extinct (both in isolation and as a part of mixed populations) or showed clear positive growth between transfers. The *C. reinhardtii* populations were scored as 'rescued' if they repeatedly scored the same or higher number of cells / ml each time sampled (minimum three consecutive growth cycles), thus showing positive growth in lethal conditions. The populations were scored as 'extinct' if no cells were detected after both visual inspection of liquid culture (under inverted microscope with 20 times magnification) and incubation of a culture sample (200 µl, approximately 10% of a culture) plated on a Petri dish with agar.

3. Data analysis

The probability of evolutionary rescue was estimated by fitting a Generalised Linear Model (binomial logistic regression) with two factors as categorical independent variables ('mode of reproduction' and 'competition'), and survival/extinction as a binary response variable. The factors were treated as fixed effects. 'Mode of reproduction' comprises two levels (sexual or asexual populations), whilst 'competition treatment' comprises five levels: absence of the competitor and each of the four wild types being considered as a different level. Since our primary question is about the interaction between mode of reproduction and competition, this two-way interaction was also included in the model. Since a binomial logistic regression is fitted on a log-odds scale, this interaction tests whether the relative effect of sex on the odds of rescue depends on whether competition is present or absent. Based on a significant overall effect of the competition treatment, this effect was further explored by model simplification (Crawley, 2013). Since our main objective was to investigate the differences between the absence and presence of the competitor, the full model was simplified by stepwise pooling the levels within 'competition treatment' factor

corresponding to the individual wild types (starting by pooling the two competitors that have caused most extinctions) until the point which further simplification was not statistically significant.

The adaptedness of survived populations was analyzed by fitting Two-Way ANOVA, with two factors as categorical independent variables ('mode of reproduction' and 'competition') and 'mean population size per growth cycle' as a continuous response variable. The populations which survived in the presence of the competitor (irrespective of the wild type) were pooled into a single level, since no evolutionary rescue events were recorded in some treatment groups. Hence, 'competition' comprises two levels: presence and absence of the competitor. The data was rank transformed prior to the analysis.

The difference in the probability of survival between *C. reinhardtii* and *C. moewusii*, while being propagated in mixed populations, was tested by fitting a Generalised Linear Model (binomial logistic regression) with 'species' as categorical independent variable. The difference in the probability of survival among different *C. moewusii* wild types was tested by fitting a Generalised Linear Model (binomial logistic regression) with 'wild type' and 'mode of reproduction (of *C. reinhardtii*)' as categorical independent variables.

The difference in high salt sensitivity between *C. reinhardtii* ancestral populations and *C. moewusii* was tested by fitting One-Way ANOVA, with 'species' as categorical independent variable. The dependent continuous variable was the slope of the curve representing mean change of a population size (yield) as a function of time (h).

All the analyses were performed using R (R core team, 2017).

4. Results

Evolutionary rescue

Overall, sex increased the probability of evolutionary rescue (Binary logistic regression; $\chi^2 = 5.82$; $df = 1$; $P = 0.016$). Of the initial 48 populations for each mode of reproduction, 19 sexual populations survived the lethal treatment (40%); in contrast, only 9 asexual populations (19%) avoided extinction (Figure 2A). Competition treatment affected the probability of evolutionary rescue (Binary logistic regression; $\chi^2 = 10.3$; $df = 4$; $P = 0.005$). Rescue was higher in the treatment with no competitor (42%) than in the 4 treatments containing a competitor (33%, 25%, 8% and 0% populations survived propagated with cc-1420, cc-1481, cc-1480 and cc-1419, respectively). The best fit was obtained by pooling the three levels (corresponding to the three wild types of the competitor that caused most extinctions) of 'competition treatment' factor. Hence, the final (minimum adequate) model comprised the factor 'competition treatment' with three levels. The model simplification suggested that the difference between the treatments with the competitor and no competition treatment was significant for three of the competitor wild types: cc-1480, cc-1419 or cc-1481, where rescue was substantially lower (Figure 2C).

However, whilst competition reduced evolutionary rescue in most of the treatments, the benefit of sex was not affected by the presence of a competitor. Sexual populations had the higher probability of evolutionary rescue both in the presence and absence of the competitor (Figure 2B) and there was no evidence of an interaction between mode of reproduction and competition treatment (Binary logistic regression; $\chi^2 = 8.0$; $df = 4$; $P = 0.09$).

C. moewusii had significantly higher probability of survival relative to *C. reinhardtii*, since it went extinct in only 6 mixed populations (12%) (Binary logistic regression; $\chi^2 = 53.49$; $df = 1$; $P < 0.0001$). The probability of survival of *C. moewusii* was contingent on the wild type (Binary logistic regression; $\chi^2 = 10.08$; $df = 3$; $P = 0.02$): cc-1481 and cc-1420 have gone

extinct in 33% and 17% of mixed populations, respectively; no extinctions were recorded for cc-1480 and cc-1419 wild types. The probability of survival of *C. moewusii* did not depend on mode of reproduction of *C. reinhardtii* (Binary logistic regression; $\chi^2 = 0.74$; df = 1; P = 0.39).

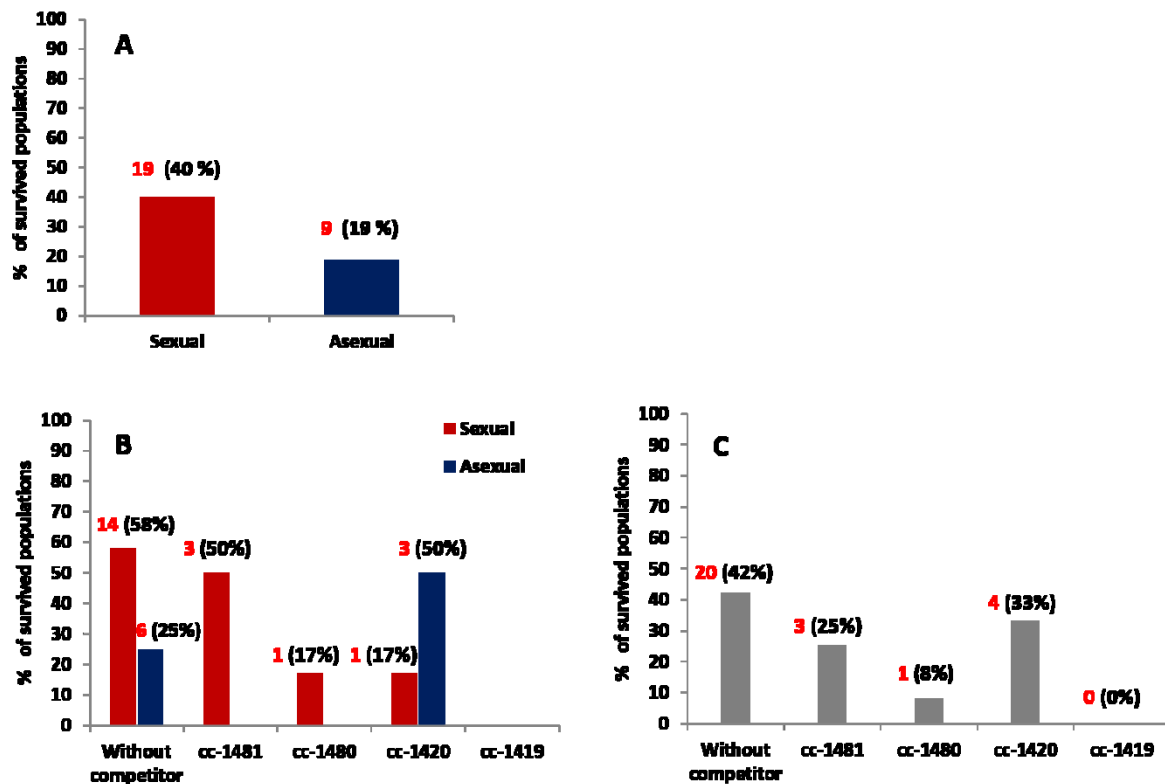


Figure 2 – Probability of evolutionary rescue for experimental populations of the focal species: for each mode of reproduction (A), with respect to both mode of reproduction and the competition treatment (B); with respect to the competition treatment (C); percentage of rescued populations per each treatment group is plotted on the Y axis; the number of survived populations per each treatment group is presented above each bar plot, with percentage of survived populations of the initial number of experimental populations in brackets; the initial number of experimental populations was 48 for each mode of reproduction and 48 for each treatment group propagated with or without the competitor, with equal number of populations (12) propagated with one of the four wild types of the competitor.

Adaptedness of rescued populations

In order to investigate the effect of mode of reproduction and competition treatment on adaptedness of survived experimental populations of the focal species, we estimated population size after each growth cycle by counting the colonies appeared on agar plates after 4 days. Sex affected adaptedness of survived experimental populations (Two-way ANOVA; $F_{1,27} = 5.09$; $P = 0.03$) (Figure 3A). Sexual populations which survived the lethal treatment had more than four-fold advantage over asexual populations with respect to population size. There was no evidence for any effect of the competition treatment on mean population size of survived experimental populations (Two-way ANOVA; $F_{1,27} = 0.04$; $P = 0.83$) (Figure 3B). There was no interaction between mode of reproduction and competition on adaptedness of survived populations (Two-way ANOVA; $F_{1,27} = 0.89$; $P = 0.35$). Survived sexual populations had higher mean population size than asexual populations, irrespective of the presence of the competitor (Figure 3C).

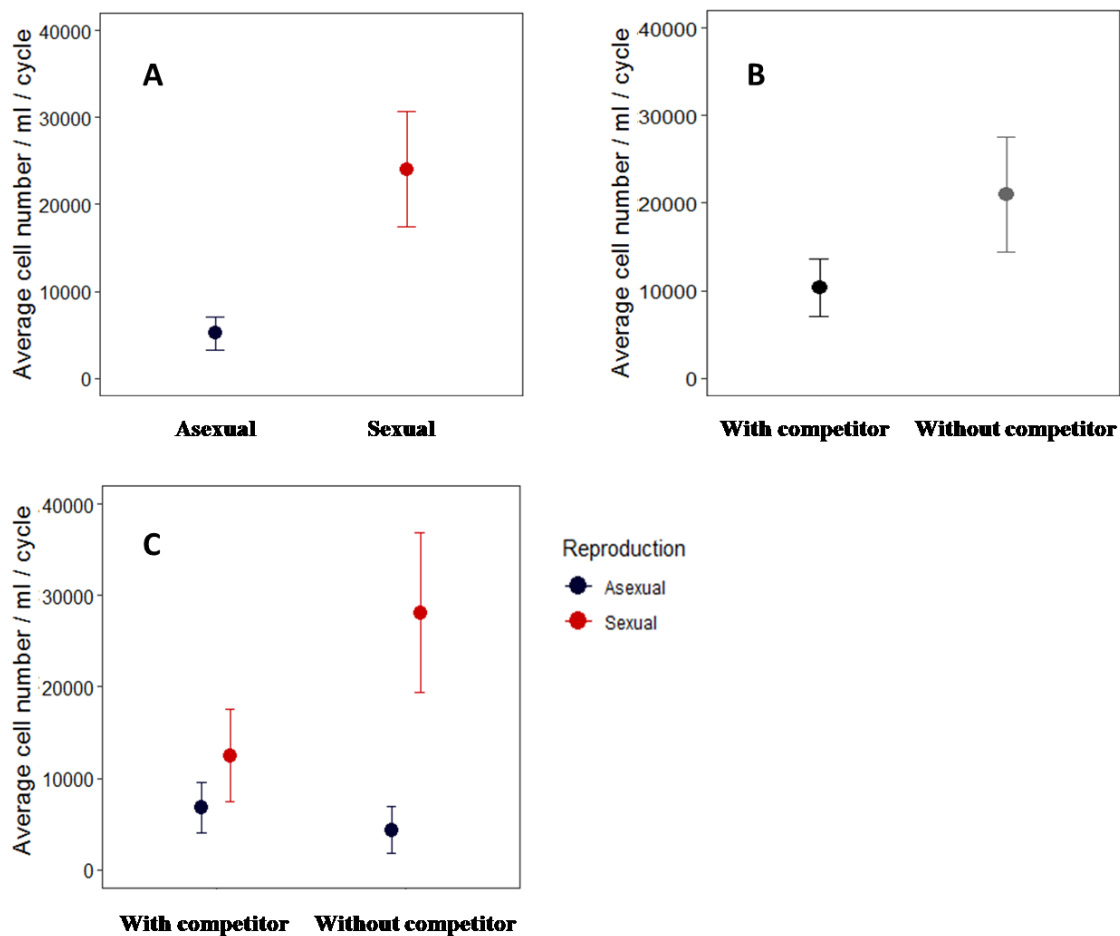


Figure 3 - Mean population size of survived experimental populations of the focal species: for each mode of reproduction (A); with respect to competition treatment (B); with respect to both mode of reproduction and competition treatment (B); the bars represent standard error of the mean.

Assay of experimental species for high salt tolerance

The growth rate of both species in the high salt environment, measured as the slope of the line representing mean change of a population size (yield) as a function of time, was significantly different (One-Way ANOVA; $F_{1,42} = 13.85$; $P = 0.0006$) (Figure 4). The average growth of *C. moewusii* wild type isolates was positive, despite considerable reduction in maximal yield in comparison to a benign medium (by 89%, data not shown). By contrast, the average growth of *C. reinhardtii* ancestral isolates was negative.

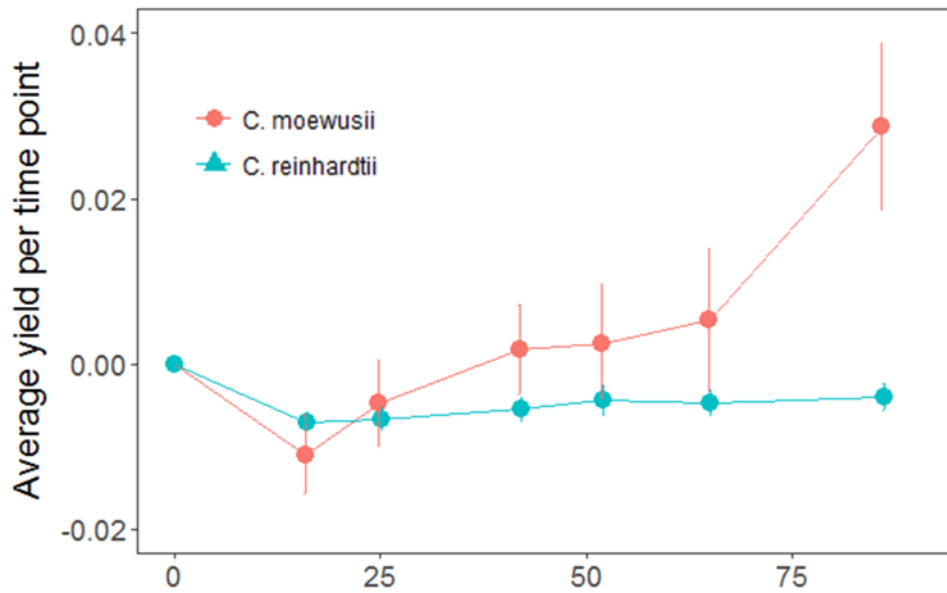


Figure 4 - The average yield of *C. reinhardtii* ancestral isolates (blue) and *C. moewusii* wild type isolates (red) per each time point (h), measured in the high salt medium (Bold's supplemented with 15 g/l of salt); the bars represent standard error of the mean.

5. Discussion

Previous studies have shown that sex can be beneficial when an environment deteriorates in a simple way (Lachapelle and Bell, 2012; Lachapelle, 2015). Our findings are consistent with these results, given that relative advantage of sexual over asexual populations (in the number of evolutionary rescue events) was 57% in an environment comprising a deteriorating abiotic component. To the best of our knowledge, this is the first study to examine how the advantageous effects of sex are affected by the presence of a competitor species. The results of our experiment indicate that sex remains beneficial in the presence of a competitor, given that sexual populations maintained the relative advantage in the number of evolutionary rescue events (40% higher than that of asexual populations). The advantage of sex is also reflected by the larger population sizes of sexual populations that avoided extinction, both in the presence and absence of the competitor. Furthermore, we

found no evidence that the size of the advantage of sex was affected by the presence of the competitor, given the absence of interaction between mode of reproduction and competition treatment.

The plausible explanation for the overall benefits of sex is a higher diversity of favorable genotypes (with higher absolute fitness in stressful conditions) generated by sex before the conditions have become lethal, which increased the efficiency of selection. These genotypes may have risen in frequency and thus positively affected mean population size. Since population size determines the supply of mutations beneficial in changing conditions (Samani and Bell, 2010), sexual populations may have been more likely to adapt and survive when conditions have become lethal.

We suggested three possible outcomes of interplay between sex and competition: the costs of sex may be augmented by competition, resulting in overall disadvantage of sex (the sign of the effect changes); sex may remain beneficial in the presence of competitor, but the level of benefits may decline due to the negative effects of competition (the magnitude of the effect decreases); the advantage of sex may increase with an increase of environmental complexity (the magnitude of the effect increases). The lack of evidence of any interaction between mode of reproduction and the competition treatment seemingly rules out all three of these predictions. However, the actual difference in the relative survival of sexual populations observed in our study was reasonably large (2.33 times the asexual survival probability in the absence of the competitor and 1.66 times in the presence of the competitor), suggesting there may have been differences that we did not have the power to detect with our study. Thus, while our results allow us to reasonably confidently rule out competition either substantially enhancing benefits of sex, or causing sex to become detrimental, we cannot completely rule out competition causing a moderate reduction in benefits of sex.

Whilst there is evidence that the advantage of sex increases with an increase of complexity of novel abiotic environment (Kaltz and Bell, 2002; Luijckx et al., 2017) due to faster assembling of multilocus genotypes, no such change of the effect was detected in the presence of a competitor. This is surprising, since adaptation to the abiotic and biotic component of novel environment may involve selection acting on different sets of traits (Collins, 2011), which are likely to be controlled by multiple loci. The negative effect of competition may manifest through reduction of population size, as demonstrated in the experiments carried out in benign environments (Ayala 1969; Bengtsson 1989; Martin and Martin, 2001). If the major negative effect of competition in our experiment was reduction of population size of a focal species, the further increase of beneficial effect of sex with an increase of environmental complexity (incorporation of the competitor into the environment) may have been hampered by the limitation of favorable genotypes.

The overall effect of competition on the probability of evolutionary rescue was negative. The focal species was driven to extinction in 83% of all the mixed populations, compared to 58% probability of extinction in the absence of the competitor. Moreover, negative effect of competition was consistent for all four wild types of the competitor (though statistically significant for only three of them). Even though the probability of survival of *C. moewusii* varied among the wild types, each wild type was more likely to survive than the focal species and caused reduction of *C. reinhardtii* survival probability relative to the group propagated in the absence of the competitor, which ranged from 9% (cc-1420) to 42% (cc-1419). A lower sensitivity of the competitor to the elevated salt concentration, manifested through a faster growth rate in the high salt medium is a plausible explanation for reduced probability of survival of the focal species in the presence of *C. moewusii*.

For the interpretation of the results of our experiment, it is important to take into account the possibility that survival of the populations of the focal species was due to competitive exclusion of the competitor, without adaptive evolution. Given a higher competitiveness of *C. moewusii* in the high salt medium (positive growth contrasted by

negative growth of *C. reinhardtii*), we rule out the possibility that survival of *C. reinhardtii* can be attributed solely to competitive exclusion of *C. moewusii*. Since all the ancestral isolates of our experimental populations showed negative growth in the high salt environment, any recorded survival has to be the result of adaptive evolution (i.e. evolutionary rescue), which may have then caused competitive exclusion of *C. moewusii*. We also rule out the possibility that survival of *C. reinhardtii* populations was facilitated by the potential elimination of *C. moewusii* from mixed populations in the early stages of experiment. Along with the advantage of faster (and qualitatively different) growth, the competitor species rarely went extinct throughout the course of the experiment (in only 12% of mixed populations), so we can conclude that competition persisted for most populations throughout most of the experiment.

Our experiment provided evidence that sex may be the beneficial strategy in the presence of the competitor (though the magnitude of the effect may potentially decrease), despite overall negative effect of competition. Given that competitive interactions are widespread in nature, one of the explanations for the maintenance of sex by natural selection may be the increased rate of adaptation of sexual populations both in the presence and absence of competitors. An outstanding question is: will the effects of sex change if a degree of complexity of environmental change further increases (with an increase of a number of competitors)? A population size of adapting population may decline more prominently in the presence of more competitors. With a lower supply of beneficial mutations as a consequence, beneficial effect of sex may diminish. However, experimental evidence is required.

Will the effect of competition be altered by differences in sensitivity of competing species to a stressor imposed on them? In our experiment, *C. moewusii* showed higher degree of tolerance to elevated salt concentration than the focal species. However, the negative effects of competition may be reduced if the difference between the competitors

with respect to maladaptedness (sensitivity) to a particular stressor is smaller. However, the experiments with direct manipulation of this factor are required. Alternatively, the probability of evolutionary rescue may be affected by other factors, such as ecological characteristics of a competitor. Recent studies suggest that the negative effect of competition is proportional to the niche overlap between competitors (Osmond and de Mazancourt, 2013). Two ecologically separated competitors would potentially suffer a lower population decline than the ones characterized by the similar patterns of resource use. Similarly, the phylogenetic distance between competitors may indirectly influence the likelihood of evolutionary rescue, under the hypothesis that the level of competition is directly proportional to relatedness between competitors (Naughton et al., 2015). However, *C. moewusii* and *C. reinhardtii* occupy different ecological niches, the former being a freshwater organism and the latter terrestrial. Moreover, both species are on the opposite ends on the phylogenetic tree of *Chlamydomonas* genus, thus being distantly related. This indicates that the effect of competition on evolutionary rescue may remain negative, regardless of the identity of the competitor. Whether this is the widespread pattern in nature, remains to be investigated.

Data accessibility

Data will be available through the Dryad Digital Repository (datadryad.org).

Conflict of interest

The authors declare no conflict of interest.

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